



HAL
open science

PCB-induced endothelial cell dysfunction: role of poly (ADP-ribose) polymerase

Simon G. Helyar, Bella Patel, Kevin Headington, Mary El-Assal, Prabal K. Chatterjee, Pal Pacher, Jon G. Mabley

► To cite this version:

Simon G. Helyar, Bella Patel, Kevin Headington, Mary El-Assal, Prabal K. Chatterjee, et al.. PCB-induced endothelial cell dysfunction: role of poly (ADP-ribose) polymerase. *Biochemical Pharmacology*, 2009, 78 (8), pp.959. 10.1016/j.bcp.2009.06.019 . hal-00514600

HAL Id: hal-00514600

<https://hal.science/hal-00514600>

Submitted on 3 Sep 2010

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

Accepted Manuscript

Title: PCB-induced endothelial cell dysfunction: role of poly (ADP-ribose) polymerase

Authors: Simon G. Helyar, Bella Patel, Kevin Headington, Mary El-Assal, Prabal K. Chatterjee, Pal Pacher, Jon G. Mabley



PII: S0006-2952(09)00488-2
DOI: doi:10.1016/j.bcp.2009.06.019
Reference: BCP 10228

To appear in: *BCP*

Received date: 13-4-2009
Revised date: 11-6-2009
Accepted date: 15-6-2009

Please cite this article as: Helyar SG, Patel B, Headington K, El-Assal M, Chatterjee PK, Pacher P, Mabley JG, PCB-induced endothelial cell dysfunction: role of poly (ADP-ribose) polymerase, *Biochemical Pharmacology* (2008), doi:10.1016/j.bcp.2009.06.019

This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

1
2
3 **PCB-induced endothelial cell dysfunction: role of poly (ADP-ribose) polymerase.**
4
5

6
7 Simon G. Helyar¹, Bella Patel², Kevin Headington², Mary El-Assal², Prabal K. Chatterjee²,
8
9 Pal Pacher³, and Jon G. Mabley²
10
11

12
13
14 ¹Brighton and Sussex Medical School, Falmer, Brighton, BN1 9PH, UK
15

16
17 ²School of Pharmacy and Biomolecular Sciences, University of Brighton, Cockcroft
18
19 Building, Brighton, BN2 4GJ, UK
20

21
22 ³Section on Oxidative Stress Tissue injury, Laboratory of Physiological Studies, National
23
24 Institutes of Health/NIAAA, 5625 Fishers lane, Bethesda, MD 20892-9413, USA
25
26

27
28
29 **Keywords:** polychlorinated biphenyl; oxidative stress; PARP; cardiovascular;
30
31 atherosclerosis.
32
33

34
35
36 **Author of correspondence:** Dr Jon Mabley
37

38 School of Pharmacy and Biomolecular Sciences,
39

40 University of Brighton,
41

42 Cockcroft Building,
43

44 Lewes Road,
45

46 Brighton BN2 4GJ, UK
47

48 E.mail: j.g.mabley@brighton.ac.uk
49

50
51
52 Tel: +44 (0) 1273 642055
53

54
55 Fax: +44 (0) 1273 679333
56
57
58
59
60

Abstract

Polychlorinated biphenyls (PCBs) are persistent environmental pollutants implicated in the development of pro-inflammatory events critical in the pathology of atherosclerosis and cardiovascular disease. PCB exposure of endothelial cells results in increased cellular oxidative stress, activation of stress and inflammatory pathways leading to increased expression of cytokines and adhesion molecules and ultimately cell death, all of which can lead to development of atherosclerosis. To date no studies have been performed to examine the direct effects of PCB exposure on the vasculature relaxant response which if impaired may predispose individuals to hypertension, an additional risk factor for atherosclerosis. Overactivation of the DNA repair enzyme poly (ADP-ribose) polymerase (PARP) following oxidative/nitrosative stress in endothelial cells and subsequent depletion of NADPH has been identified as a central mediator of cellular dysfunction. The aim therefore was to investigate whether 2,2',4,6,6'-pentachlorobiphenyl (PCB 104) directly causes endothelial cell dysfunction via increased oxidative stress and subsequent overactivation of PARP. Exposure of *ex vivo* rat aortic rings to PCB 104 impaired the acetylcholine-mediated relaxant response, an effect that was dependent on both concentration and exposure time. *In vitro* exposure of mouse endothelial cells to PCB 104 resulted in increased cellular oxidative stress through activation of the cytochrome p450 enzyme CYP1A1 with subsequent overactivation of PARP and NADPH depletion. Pharmacological inhibition of CYP1A1 or PARP protected against the PCB 104-mediated endothelial cell dysfunction. In conclusion, the environmental contaminants, PCBs, can activate PARP directly impairing endothelial cell function that may predispose exposed individuals to development of hypertension and cardiovascular disease.

1. Introduction

Polychlorinated biphenyls (PCBs) belong to a class of widely dispensed and persistent environmental contaminants collectively referred to as halogenated aromatic hydrocarbons. The commercial production of these compounds was banned at the end of the 1970s but PCBs continue to be a health problem partly due to their formation as industrial by-products but mainly because of their persistence in the environment and our food chain [1, 2]. The major source of exposure to PCBs in the general population is from dietary intake of contaminated foods that include fish, meat and dairy products. Epidemiology studies have demonstrated that cardiovascular diseases can be linked to environmental pollution. Human exposure to PCBs for example, among Swedish capacitor manufacturing workers and the Italian population of Serveso (exposed to PCBs as a result of an industrial accident in 1976), has resulted in a detectable increase in cardiovascular disease [3].

Exposure to PCBs can lead to cardiovascular toxicity and atherosclerosis in animal models [4] as well as having a pro-inflammatory effect on endothelial cells [5]. PCB-mediated dysfunction in the vascular endothelium has been linked to increased oxidative stress mediated through the activation of a cytochrome P450 oxidase, CYP1A1, following PCB activation of the Ah receptor [5-7] and mediated by caveolae signalling [8]. PCB-mediated cellular oxidative stress leads to subsequent activation of stress kinases [9], increased transcription of NF- κ B [7], increased pro-inflammatory gene expression (cytokines, adhesion molecules) [10], disruption of endothelial cell barrier function [11], and endothelial cell apoptosis [12], all of which can lead to atherosclerosis [5, 13].

1
2
3 In the early 1980's it was suggested that PCB exposure was linked to
4
5 hypertension [14] and this has been confirmed with much larger population studies [15,
6
7 16]. Hypertension is a contributing factor for development of atherosclerosis and though
8
9 PCBs have already been shown to exert a pro-inflammatory effect on endothelial cells
10
11 increasing the risk of developing atherosclerosis no studies on the direct effects of PCBs on
12
13 endothelial cell function have been performed.
14
15

16
17 Endothelial dysfunction is commonly associated with pathology of the
18
19 cardiovascular system where overproduction of reactive oxygen and nitrogen intermediates
20
21 in endothelial cells leads to DNA damage and overactivation of a DNA repair enzyme, poly
22
23 (ADP-ribose) polymerase (PARP), representing a final common pathway in the
24
25 pathogenesis of many cardiovascular diseases. PARP activation has been implicated as a
26
27 central mediator of endothelial cell dysfunction in a variety of disease states including
28
29 diabetes [17, 18], atherosclerosis [19], ageing [20], doxorubicin-induced dysfunction [21]
30
31 and septic shock [22].
32
33
34
35

36 Therefore the aim of this study was to examine the direct effects of 2,2',4,6,6'-
37
38 pentachlorobiphenyl (PCB 104, Fig 1), an example of a highly ortho-chlorinated nonplanar
39
40 PCB, on endothelial cell function and investigate whether increased cellular oxidative stress
41
42 and activation of PARP is responsible for any deleterious effects.
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60
61
62
63
64
65

2. Materials and Methods

2.1 Reagents

Acetylcholine, α -naphthoflavone, 2,3-bis(2-methoxy-4-nitro-5-sulphophenyl)-2H-tetrazolium-5-carboxanilide (XTT), dicumarol, digitonin, dimethyl sulphoxide (DMSO), endothelial cell growth supplement, 7-ethoxyresorufin, heparin, 1-methoxy-5-methylphenazinium, nitro blue tetrazolium (NBT), phenylephrine, resorufin, sodium dodecyl sulfate (SDS), sodium nitroprusside and thiazolyl blue tetrazolium bromide (MTT) were obtained from Sigma/Aldrich (Poole, UK). $^3\text{H-NAD}$ was obtained from PerkinElmer Ltd. (Windsor, UK). PJ-34 was purchased from Calbiochem (Nottingham, UK). Hams F12 culture media, penicillin, streptomycin and fetal bovine serum (FBS) were obtained from Lonza (Nottingham, UK). Sprague-Dawley rats used in the *ex vivo* studies were obtained from Charles River Laboratories (Kent, UK). PCB-104 (purity 99.1%) was obtained from QMX laboratories (Essex, UK). All other chemicals were of reagent grade and obtained from Fisher Scientific (Loughborough, UK).

2.2 *Ex vivo*

Thoracic aorta from male Sprague-Dawley rats (180-220g) were dissected and cleared of periadventitial fat and connective tissue. Rings of 2-3mm were cut and placed into Hams F12 medium supplemented with 3% FBS, endothelial cell growth supplement (0.03 mg/ml), heparin (50U/ml) and 1% penicillin and streptomycin. PCB 104 was dissolved in DMSO to make a stock solution which was warmed to 37°C before being added to pre-warmed media at 37°C at the appropriate volumes to obtain concentrations of 1, 3, 10 and 20 μM . For the initial experiments rings were incubated with Hams F12 media

1
2
3 alone (control) or with PCB 104 1, 3 10 or 20 μ M, for 4 or 6h at 37°C with a 95% O₂, 5%
4
5 CO₂ mix. Subsequent to these experiments rings were exposed to PCB 104 10 μ M in
6
7 combination with inhibitors of CYP 1A1 (α -naphthoflavone 0.3 μ M) and PARP (PJ-34
8
9 3 μ M) for 4h.

10
11
12 Subsequent to the relevant incubation the aortic rings were mounted in organ baths
13
14 filled with warmed (37°C) and gas-equilibrated (95% O₂, 5% CO₂) Krebs solution
15
16 containing (in mmol/L) CaCl₂ 1.6, MgSO₄ 1.17, EDTA 0.026, NaCl 130, NaHCO₃ 14.9,
17
18 KCl 4.7, KH₂PO₄ 1.18, and glucose 5. Isometric tension of the rings was measured with
19
20 isometric transducers (DMT), digitised using PowerLab and displayed on a Macintosh
21
22 computer. A preload tension of 1.5g was applied and the rings were equilibrated for 60 min,
23
24 followed by measurements of the concentration-dependent contraction to phenylephrine
25
26 (10⁻⁹ to 10⁻⁴ M) and in rings precontracted with phenylephrine (10⁻⁶M), relaxation to
27
28 acetylcholine (10⁻⁹ to 10⁻⁴M) and sodium nitroprusside (10⁻¹² to 10⁻⁵ M).
29
30
31
32
33
34
35

36 2.3 *In vitro*

37
38 Mouse aortic endothelial cells [17] were plated at a density of 250,000 cells per well
39
40 in a 12 well plate and grown in Ham's F12 media supplemented with 10% fetal calf serum,
41
42 heparin (50U/ml) and 0.03 mg/ml Endothelial Cell Growth Supplement. Following 24h the
43
44 media was replaced and the cells treated with increasing concentrations of PCB 104 (1, 3,
45
46 10 or 0 or 20 μ M) for 2, 4 or 6h. In a second set of experiments mouse endothelial cells
47
48 were treated with 10 μ M PCB 104 in combination inhibitors of CYP 1A1 (α -
49
50 naphthoflavone 0.3 μ M) and PARP (PJ-34 3 μ M) for 4h. The following measurements
51
52 were then determined.
53
54
55
56
57
58
59
60
61
62
63
64
65

2.4 Oxidative stress

Cellular levels of oxidative stress were assessed by reduction of nitro blue tetrazolium (NBT) as previously described [23]. Briefly, mouse endothelial cells were grown in 12 well plates and incubated at 37 °C with PCB 104 ± pharmacological inhibitors for 2, 4 or 6h in the presence of 25 µg/ml NBT. Following the incubation period the media was removed from the wells and replaced with 75% ethanol for 10 minutes then removed. The wells were then washed twice with 100% methanol followed by air-drying of the wells. The blue crystals were then solubilised in a mixture of potassium hydroxide and DMSO (5:6) before absorbance was measured at 700 nm and results expressed as a percentage of absorbance in untreated cells.

2.5 CYP1A1 activity

The activity of CYP1A1 was assayed by the o-dealkylation of ethoxyresorufin (EROD) [24]. Briefly, the media was removed and replaced with 250 µl fresh media containing 8 µM 7-ethoxyresorufin and 10 µM dicumarol for an additional 30 min incubation at 37 °C. Following the incubation period 100 µl was removed and mixed with 130 µl of absolute ethanol. Resorufin-associated fluorescence was measured in a multiwell fluorescence reader, with excitation/emission wavelengths of 530/590 nm and compared to a resorufin standard curve. Cellular protein was determined using the Bradford assay [25] and results expressed as pmol resorufin/mg protein/min.

2.6 PARP activity

PARP activity was measured as previously described [26]. Briefly, the media was removed and replaced with 0.5 ml HEPES (pH 7.5) containing 0.01% digitonin and ^3H -NAD ($0.5 \mu\text{Ci ml}^{-1}$) and the cells incubated for 20 min at 37°C . The cells were then scraped from the wells and placed in an Eppendorf tubes containing 200 μl of ice-cold 50% TCA (w/v), the tubes were then placed at 4°C . After 4h the tubes were centrifuged at 1800g for 10 min and the supernatant removed, the pellet was washed twice with 500 μl ice-cold 5% TCA. The pellet was solubilized in 250 μl NaOH (0.1M) containing 2% SDS overnight at 37°C , the PARP activity was then determined by measuring the radioactivity incorporated using a Wallac scintillation counter. The solubilised protein (250 μl) was mixed with 5 ml of scintillant (ScintiSafe Plus, Fisher) before being counted for 10min. Results are expressed as a percentage of the PARP activity observed in untreated cells.

2.7 NADPH depletion

Cell NADPH levels were determined as previously described [27]. Briefly, the media was removed and the cells washed with once with PBS before 100 μl of fresh media containing 0.5 mM 2,3-bis(2-methoxy-4-nitro-5-sulfohenyl)-2H-tetrazolium-5-carboxanilide (XTT) and 20 nM 1-methoxy-5-methylphenazinium was added to each well. The plate was then incubated for 30 min and absorbencies were read at 450 and 670 nm. Results were expressed as a percentage of the absorbance observed in untreated cells.

2.8 MTT assay

1
2
3 Cell viability was determined by the reduction of yellow MTT into a purple
4 formazan product by mitochondrial dehydrogenases of metabolically active cells.
5 Following the treatment period the experimental media was removed and 200 μ l MTT (1
6 mg/ml) added. After 1h the MTT solution was carefully removed and the purple crystals
7 were solubilized in 100 μ l of DMSO. The DMSO was transferred to an ELISA plate and
8 absorbance measured at 550 nm with a 620 nm reference [26]. Results were expressed as a
9 percentage of the absorbance observed in untreated cells.
10
11
12
13
14
15
16
17
18
19
20
21

22 *2.9 Statistical analysis*

23
24 Results are presented as mean \pm standard error of the mean (SEM). Analysis of
25 variance with Bonferroni's correction or Student's t-test was used to compare mean values
26 as appropriate. Differences were considered significant when $p < 0.05$.
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60
61
62
63
64
65

3. Results

3.1 PCB 104 exposure results in dose- and time-dependent endothelial cell dysfunction

The effect of short-term exposure to PCB 104 on rat endothelial cell function was investigated by exposing aortic rings to PCB 104 1, 3, 10 or 20 μM for 2, 4 or 6h. Endothelial cell function was assessed by the ability of the endothelial cell to produce nitric oxide in response to acetylcholine and cause subsequent vascular smooth muscle cell relaxation.

Acetylcholine-mediated relaxation of rat aortic rings is impaired following exposure to PCB 104 at 3, 10 or 20 μM (Fig 2a and b) for 4 or 6h. There was a significant increase in the EC50 concentration of acetylcholine following PCB exposure as compared to untreated rings (Table 1), however though the mean EC50 increases both with higher doses and longer exposure time this did not reach statistical significance (Table 1). There was no effect on endothelial cell function following 2h exposure to PCB 104 at any of the three concentrations tested (data not shown). In addition, exposure to 1 μM PCB 104 had no effect on endothelial cell function at any exposure time tested (data not shown). This impairment of endothelial cell function was both dose dependent and time dependent particularly at the higher, 10 and 20 μM , concentrations of PCB 104. PCB 104 exposure had no effect on vascular smooth muscle contraction to phenylephrine or on relaxation in response to sodium nitroprusside (data not shown).

3.2 PCB 104 exposure increases endothelial cell oxidative stress, CYP1A1 activity, and PARP activity while depleting NADPH levels.

1
2
3 To investigate the mechanism by which PCB 104 may be causing cell dysfunction
4
5 mouse endothelial cells were exposed to PCB 104 *in vitro* and cellular levels of oxidative
6
7 stress, CYP1A1 activity, PARP activity and NADPH were measured.
8
9

10 PCB 104 between 1 and 10 μM dose and time dependently increased endothelial
11
12 cell oxidative stress (Fig 3a) as measured by NBT reduction. This increased oxidative
13
14 stress following PCB 104 exposure is likely mediated by increased CYP1A1 activity.
15
16 Exposure of endothelial cells to PCB 104 dose- and time-dependently increased CYP1A1
17
18 activity as measured by EROD activity (Fig 3b). Although 2h exposure to 20 μM PCB 104
19
20 increases both oxidative stress and EROD activity (Fig 3a and b), there is, however, a
21
22 significant decrease in the measured oxidative stress and EROD activity after 4 or 6 h
23
24 exposure at this PCB 104 concentration that may indicate that at this very high
25
26 concentration of PCB 104 either CYP1A1 becomes inhibited or the endothelial cells
27
28 themselves are becoming metabolically dysfunctional.
29
30
31

32
33
34 PCB 104 exposure dose-and time-dependently increased PARP activity as measured
35
36 by NAD incorporation (Fig 3c). As NAD is the substrate for PARP, depletion of NAD
37
38 related high-energy phosphate levels following PARP overactivation has been identified as
39
40 a mechanism of cellular dysfunction, particularly in endothelial cells where NADPH is an
41
42 essential co-factor for nitric oxide synthase and subsequent nitric oxide production. PCB
43
44 104 exposure significantly depleted endothelial cell NADPH levels (Fig 3d) an effect that
45
46 appears to be linked to PARP activity as the higher the PARP activity the more cellular
47
48 NADPH is depleted (Fig 3c and d).
49
50
51
52
53
54
55
56
57
58
59
60
61
62
63
64
65

1
2
3 *3.3 Inhibition of PARP and CYP1A1 protects against PCB-104-mediated endothelial cell*
4
5 *dysfunction.*
6

7 To determine whether PCB 104-mediated activation of CYP1A1 and PARP
8 mediates the observed endothelial cell dysfunction we pharmacologically inhibited
9 CYP1A1 with α -naphthoflavone [28] and PARP with PJ 34 [29].
10
11
12
13

14 Exposure to 10 μ M PCB 104 for 4h significantly impaired endothelial cell function
15 as assessed by acetylcholine-mediated vascular ring relaxation (Fig 4) and increased the
16 EC50 for acetylcholine from $1.2 \times 10^{-7} \pm 0.2 \times 10^{-7}$ M to $5.0 \times 10^{-7} \pm 1 \times 10^{-7}$ M ($p < 0.05$). Inhibition
17 of PARP with PJ-34 (3 μ M) or CYP1A1 activity with α -naphthoflavone (0.3 μ M)
18 completely protected against the PCB 104-mediated endothelial cell dysfunction (Fig 4) as
19 well as returning the EC50 for acetylcholine to $0.6 \times 10^{-7} \pm 0.2 \times 10^{-7}$ M and $0.8 \times 10^{-7} \pm 0.3 \times 10^{-7}$
20 M respectively ($p < 0.05$ vs. PCB 104 10 μ M). Neither PJ-34 nor α -naphthoflavone alone
21 had any effect on vascular ring contraction induced by phenylephrine or relaxation by
22 acetylcholine (data not shown).
23
24
25
26
27
28
29
30
31
32
33
34
35

36 To confirm that both these pharmacological inhibitors were affecting the cellular
37 activities of PARP and CYP1A1 we carried out *in vitro* experiments exposing endothelial
38 cells to 10 μ M PCB 104 for 4h and measuring oxidative stress, CYP1A1 activity, PARP
39 activity and NADPH depletion.
40
41
42
43
44

45 PCB 104 exposure significantly increased endothelial cell oxidative stress (Fig 5a),
46 CYP1A1 activity (Fig 5b), PARP activity (Fig 5c) and NADPH depletion (Fig 5d). The
47 CYP1A1 inhibitor α -naphthoflavone reduced PCB 104-mediated increased EROD activity
48 to levels observed in control cells (Fig 5b) indicating that this concentration of α -
49 naphthoflavone, 0.3 μ M, was an effective inhibitory concentration. Inhibiting PCB 104-
50
51
52
53
54
55
56
57
58
59
60
61
62
63
64
65

1
2
3 induced CYP1A1 activity also prevented the increased cellular oxidative stress (Fig 5a),
4
5 PARP activity (Fig 5c) and NADPH depletion (Fig 5d).
6

7
8 The PARP inhibitor PJ-34 not only reduced the PCB 104-mediated increase in
9
10 PARP activity but also reduced the significantly reduced basal activity observed in control
11
12 cells (Fig 5c). Inhibition of PARP also significantly attenuated the NADPH depletion (Fig
13
14 5d) following PCB 104 exposure and in control cells even increased NADPH levels above
15
16 that seen in untreated cells (Fig 5d). However, inhibition of PARP had no effect on either
17
18 cellular oxidative stress or CYP1A1 activity (Fig 5a and b). Similar results were seen with
19
20 endothelial cells isolated from the PARP-1 deficient mouse where PCB-104 exposure
21
22 increased cellular oxidative stress and CYP1A1 activity levels (Fig 5a and b) but failed to
23
24 dramatically affect PARP activity or NADPH depletion (Fig 5c and d). However, in
25
26 PARP-1^{-/-} cells there was a minor increase in PARP activity and NADPH depletion that
27
28 may reflect activation of PARP-2 or other PARP isoforms, which are also present in
29
30 mammalian nuclei though at much lower levels than PARP-1. This is not observed in PJ-
31
32 34 treated endothelial cells as PJ-34 is a global PARP inhibitor and inhibits not only PARP-
33
34 1 but also PARP-2.
35
36
37
38
39
40
41
42

43 *3.4 PCB 104-mediated loss of cell viability is prevented by inhibition of CYP1A1 and* 44 45 *PARP.* 46

47
48 Exposure to PCBs has been shown to cause loss of endothelial cell viability and
49
50 increase apoptosis. Endothelial cell viability following 48h exposure to PCB 104 was
51
52 measured using the MTT assay and found to be dose dependently reduced (Fig 6).
53
54 Exposure to PCB 104 for 24h also causes loss of endothelial cell viability but only at 10
55
56
57
58
59
60
61
62
63
64
65

1
2
3 and 20 μM (data not shown). This loss of cell viability following PCB 104 exposure was
4
5 prevented by inhibition of CYP1A1, with α -naphthoflavone, or PARP, either
6
7 pharmacologically with PJ-34 or in PARP-1 gene deficient cells (Fig 6).
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60
61
62
63
64
65

4. Discussion

The data presented here demonstrates for the first time that exposure of endothelial cells to the environmental contaminant PCB 104, for a relatively short exposure period, causes direct cellular dysfunction. Serum concentrations of PCBs can reach approximately 3 μM in people exposed to these toxicants [30, 31] and possibly 10-20 μM in the extracellular space [32], it is exposure to these concentrations of PCB 104 which caused direct endothelial cell dysfunction in our study. PCB-mediated activation of the cytochrome P450 enzyme CYP1A1 and subsequently increased cellular oxidative stress appears to be the mediator of PCB-induced endothelial cell dysfunction with overactivation of the DNA repair enzyme PARP and subsequent depletion of cellular NADPH being the mechanism of dysfunction. Pharmacological inhibition of CYP1A1 or PARP protected endothelial cell function from PCB 104-mediated impairment. Previous work has established long-term exposure to PCBs as having pro-inflammatory effects on endothelial cells increasing the risk of developing atherosclerosis, our data indicates that PCBs cause direct endothelial cell dysfunction in a much shorter time period increasing the risk of developing hypertension and cardiovascular disease.

PCBs have previously been shown to increase reactive oxygen species in human breast cancer cells and cause PARP activation [33]. When PARP becomes activated, using NAD^+ as a substrate, it catalyzes the building of homopolymers of adenosine diphosphate ribose units. NAD^+ levels regulate an array of vital cellular processes, NAD^+ serves as a cofactor for glycolysis and the tricarboxylic acid cycle, thus providing ATP for most cellular processes, NAD^+ also serves as the precursor for NADP, which acts as a cofactor for the pentose shunt, for bioreductive synthetic pathways, and is involved in the

1
2
3 maintenance of glutathione (GSH) pools [34]. PCB 104 increased PARP activity in
4
5 endothelial cells and the resultant effects both on the metabolic pathways of the cell and
6
7 anti-oxidant defence systems are likely to be the major mechanism by which these
8
9 environmental contaminants cause endothelial cell dysfunction. Indeed, the cellular
10
11 glutathione status has been previously shown to modulate PCB-mediated endothelial cell
12
13 apoptosis [9] with PCB-exposure reducing endothelial cell glutathione levels and increasing
14
15 cell death, the increased PARP activation by PCBs may account for the effect on cellular
16
17 glutathione levels.
18
19
20

21
22 Activation of PARP in endothelial cells has been shown to impair relaxation of the
23
24 blood vessel in response to vasodilators such as acetylcholine [17], an effect which appears
25
26 to be mediated by a decrease in the levels of cellular high-energy phosphates and
27
28 suppression of NAD and NADPH levels [18]. Endothelial cell-mediated control of blood
29
30 pressure is via production of nitric oxide from nitric oxide synthase, an enzyme dependent
31
32 on NADPH [35]. Depletion of cellular NADPH levels in endothelial cells exposed to PCB
33
34 104 therefore is likely to result in a decreased production of nitric oxide from endothelial
35
36 cells thus reducing vasodilatation and potentially increasing the risk of developing
37
38 hypertension. This disruption of endothelial cell regulation of blood pressure by PCB 104
39
40 may account for the results observed in a recent study where high serum PCB levels in
41
42 humans were positively associated with hypertension [16]. Impaired endothelial nitric
43
44 oxide bioavailability and subsequent hypertension development is a significant risk factor
45
46 in developing atherosclerosis [36]. Indeed endothelial cell regulation of blood pressure in
47
48 the apolipoprotein E deficient mouse model of atherosclerosis, as assessed by endothelial
49
50
51
52
53
54
55
56
57
58
59
60
61
62
63
64
65

1
2
3 cell-mediated relaxation of thoracic aorta, has been shown to be significantly impaired [37,
4
5 38], with function being restored by inhibition of PARP [39].
6

7
8 Previous studies have shown PCBs to have a pro-inflammatory and hence pro-
9
10 atherosclerotic effect on endothelial cells [5, 13] coupled with our data indicating PCB-
11
12 mediated PARP activation in endothelial cells leading to cell dysfunction and pre-disposing
13
14 individuals to hypertension suggests PCB exposure poses a significant cardiovascular
15
16 health problem. The pro-inflammatory effects of PCBs on endothelial cells include
17
18 activation of stress kinases [9], increased transcription of NF- κ B [7], increased pro-
19
20 inflammatory cytokine production, increased cellular expression of adhesion molecules
21
22 [10], disruption of cell barrier function [11], and increased apoptosis [12], are all effects
23
24 which may ultimately be mediated by PCB-induced PARP activation. PARP has been
25
26 identified as having a role in atherosclerosis development [40, 41]. In animal models of
27
28 atherosclerosis PARP inhibitors have been shown to be beneficial in reducing plaque size,
29
30 increasing their stability and promoting their regression [42, 43].
31
32
33
34
35

36
37 The activation of transcription factors NF- κ B and AP-1, both upregulated in
38
39 endothelial cells by PCBs [7] and saturated fatty acids [44], has been shown to be regulated
40
41 by PARP [45, 46]. Inflammatory cytokine production, again observed in endothelial cells
42
43 following PCBs and fatty acids [13, 44], can also be blocked by PARP inhibitors [29, 47].
44
45 In diabetes [48] and atherosclerosis [49] PARP activation has been shown to mediate
46
47 endothelial cell expression of adhesion molecules such as ICAM, P-selectin and VCAM, all
48
49 of which have been implicated in the development of atherosclerosis [50] and can be
50
51 upregulated in endothelial cells following PCB exposure [10]. PARP inhibition has also
52
53 been shown to protect endothelial cell barrier function [51], which again can be damaged
54
55
56
57
58
59
60
61
62
63
64
65

1
2
3 following PCB exposure [11]. Therefore, there is a strong suggestion that PCB-mediated
4
5 PARP activation in endothelial cells may account for the pro-atherosclerotic effects of these
6
7 environmental contaminants.
8
9

10 Based on the results presented here we conclude that PCB exposure leads to
11
12 increased oxidative stress and activation of PARP in vascular endothelial cells and
13
14 impairment of vasorelaxation predisposing individuals to development of hypertension.
15
16 This vascular function impairment coupled with the previously reported pro-inflammatory
17
18 effects of PCBs on endothelial cells is likely the cause of the increased incidence of
19
20 cardiovascular disease in populations exposed to environmental contaminants such as
21
22 PCBs.
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60
61
62
63
64
65

5. References

1. Carpenter DO, Polychlorinated biphenyls (PCBs): routes of exposure and effects on human health. *Rev Environ Health* **21**(1): 1-23, 2006.
2. Turrio-Baldassarri L, Abate V, Alivernini S, Battistelli CL, Carasi S, Casella M, Iacovella N, Iamiceli AL, Indelicato A, Scarcella C and La Rocca C, A study on PCB, PCDD/PCDF industrial contamination in a mixed urban-agricultural area significantly affecting the food chain and the human exposure. Part I: soil and feed. *Chemosphere* **67**(9): 1822-30, 2007.
3. Warner M, Eskenazi B, Patterson DG, Clark G, Turner WE, Bonsignore L, Mocarelli P and Gerthoux PM, Dioxin-Like TEQ of women from the Seveso, Italy area by ID-HRGC/HRMS and CALUX. *J Expo Anal Environ Epidemiol* **15**(4): 310-8, 2005.
4. Hennig B, Reiterer G, Majkova Z, Oesterling E, Meerarani P and Toborek M, Modification of environmental toxicity by nutrients: implications in atherosclerosis. *Cardiovasc Toxicol* **5**(2): 153-60, 2005.
5. Hennig B, Meerarani P, Slim R, Toborek M, Daugherty A, Silverstone AE and Robertson LW, Proinflammatory properties of coplanar PCBs: in vitro and in vivo evidence. *Toxicol Appl Pharmacol* **181**(3): 174-83, 2002.
6. Toborek M, Barger SW, Mattson MP, Espandiari P, Robertson LW and Hennig B, Exposure to polychlorinated biphenyls causes endothelial cell dysfunction. *J Biochem Toxicol* **10**(4): 219-26, 1995.
7. Slim R, Toborek M, Robertson LW and Hennig B, Antioxidant protection against PCB-mediated endothelial cell activation. *Toxicol Sci* **52**(2): 232-9, 1999.

- 1
2
3 8. Lim EJ, Majkova Z, Xu S, Bachas L, Arzuaga X, Smart E, Tseng MT, Toborek M
4 and Hennig B, Coplanar polychlorinated biphenyl-induced CYP1A1 is regulated
5 through caveolae signaling in vascular endothelial cells. *Chem Biol Interact* **176**(2-
6 3): 71-8, 2008.
7
8
9
10
11
12 9. Slim R, Toborek M, Robertson LW, Lehmler HJ and Hennig B, Cellular glutathione
13 status modulates polychlorinated biphenyl-induced stress response and apoptosis in
14 vascular endothelial cells. *Toxicol Appl Pharmacol* **166**(1): 36-42, 2000.
15
16
17
18
19 10. Eum SY, Rha GB, Hennig B and Toborek M, c-Src is the primary signaling
20 mediator of polychlorinated biphenyl-induced interleukin-8 expression in a human
21 microvascular endothelial cell line. *Toxicol Sci* **92**(1): 311-20, 2006.
22
23
24
25
26 11. Eum SY, Andras IE, Couraud PO, Hennig B and Toborek M, Pcb's and Tight
27 Junction Expression. *Environ Toxicol Pharmacol* **25**(2): 234-240, 2008.
28
29
30
31 12. Lee YW, Park HJ, Son KW, Hennig B, Robertson LW and Toborek M, 2,2',4,6,6'-
32 pentachlorobiphenyl (PCB 104) induces apoptosis of human microvascular
33 endothelial cells through the caspase-dependent activation of CREB. *Toxicol Appl*
34 *Pharmacol* **189**(1): 1-10, 2003.
35
36
37
38
39
40
41 13. Choi W, Eum SY, Lee YW, Hennig B, Robertson LW and Toborek M, PCB 104-
42 Induced Proinflammatory Reactions in Human Vascular Endothelial Cells:
43 Relationship to Cancer Metastasis and Atherogenesis. *Toxicol Sci*, 2003.
44
45
46
47
48 14. Kreiss K, Zack MM, Kimbrough RD, Needham LL, Smrek AL and Jones BT,
49 Association of blood pressure and polychlorinated biphenyl levels. *Jama* **245**(24):
50 2505-9, 1981.
51
52
53
54
55
56
57
58
59
60
61
62
63
64
65

- 1
2
3 15. Lee DH, Lee IK, Porta M, Steffes M and Jacobs DR, Jr., Relationship between
4 serum concentrations of persistent organic pollutants and the prevalence of
5 metabolic syndrome among non-diabetic adults: results from the National Health
6 and Nutrition Examination Survey 1999-2002. *Diabetologia* **50**(9): 1841-51, 2007.
7
8
9
10
11
12 16. Everett CJ, Mainous AG, 3rd, Frithsen IL, Player MS and Matheson EM,
13 Association of polychlorinated biphenyls with hypertension in the 1999-2002
14 National Health and Nutrition Examination Survey. *Environ Res* **108**(1): 94-7,
15 2008.
16
17
18
19
20
21
22 17. Garcia Soriano F, Virag L, Jagtap P, Szabo E, Mabley JG, Liaudet L, Marton A,
23 Hoyt DG, Murthy KG, Salzman AL, Southan GJ and Szabo C, Diabetic endothelial
24 dysfunction: the role of poly(ADP-ribose) polymerase activation. *Nat Med* **7**(1):
25 108-113, 2001.
26
27
28
29
30
31 18. Soriano FG, Pacher P, Mabley J, Liaudet L and Szabo C, Rapid reversal of the
32 diabetic endothelial dysfunction by pharmacological inhibition of poly(ADP-ribose)
33 polymerase. *Circ Res* **89**(8): 684-91, 2001.
34
35
36
37
38 19. Pacher P, Mabley JG, Soriano FG, Liaudet L and Szabo C, Activation of poly(ADP-
39 ribose) polymerase contributes to the endothelial dysfunction associated with
40 hypertension and aging. *Int J Mol Med* **9**(6): 659-664, 2002.
41
42
43
44
45 20. Pacher P, Mabley JG, Soriano FG, Liaudet L, Komjati K and Szabo C, Endothelial
46 dysfunction in aging animals: the role of poly(ADP-ribose) polymerase activation.
47
48
49
50
51 *Br J Pharmacol* **135**(6): 1347-50, 2002.
52
53
54
55
56
57
58
59
60
61
62
63
64
65

- 1
2
3 21. Pacher P, Liaudet L, Bai P, Virag L, Mabley JG, Hasko G and Szabo C, Activation
4 of poly(ADP-ribose) polymerase contributes to development of doxorubicin-
5 induced heart failure. *J Pharmacol Exp Ther* **300**(3): 862-7, 2002.
6
7
8
9
10 22. Soriano FG, Liaudet L, Szabo E, Virag L, Mabley JG, Pacher P and Szabo C,
11 Resistance to acute septic peritonitis in poly(ADP-ribose) polymerase-1-deficient
12 mice. *Shock* **17**(4): 286-92, 2002.
13
14
15
16
17 23. Samai M, Hague T, Naughton DP, Gard PR and Chatterjee PK, Reduction of
18 paraquat-induced renal cytotoxicity by manganese and copper complexes of EGTA
19 and EHPG. *Free Radic Biol Med* **44**(4): 711-21, 2008.
20
21
22
23
24 24. Lai KP, Wong MH and Wong CK, Modulation of AhR-mediated CYP1A1 mRNA
25 and EROD activities by 17beta-estradiol and dexamethasone in TCDD-induced
26 H411E cells. *Toxicol Sci* **78**(1): 41-9, 2004.
27
28
29
30
31 25. Bradford MM, A rapid and sensitive method for the quantification of microgram
32 quantities of protein utilizing the principle of protein-dye binding. *Anal. Biochem.*
33 **72**: 248-254, 1976.
34
35
36
37
38 26. Mabley JG, Suarez-Pinzon WL, Hasko G, Salzman AL, Rabinovitch A, Kun E and
39 Szabo C, Inhibition of poly (ADP-ribose) synthetase by gene disruption or
40 inhibition with 5-iodo-6-amino-1,2-benzopyrone protects mice from multiple-low-
41 dose-streptozotocin-induced diabetes. *Br J Pharmacol* **133**(6): 909-919., 2001.
42
43
44
45
46
47
48 27. Nakamura J, Asakura S, Hester SD, de Murcia G, Caldecott KW and Swenberg JA,
49 Quantitation of intracellular NAD(P)H can monitor an imbalance of DNA single
50 strand break repair in base excision repair deficient cells in real time. *Nucleic Acids*
51 *Res* **31**(17): e104, 2003.
52
53
54
55
56
57
58
59
60
61
62
63
64
65

- 1
2
3 28. Bachmann K, Sanyal G, Potter J, Schiavone R and Loch J, In vivo evidence that
4 theophylline is metabolized principally by CYP1A in rats. *Pharmacology* **47(1)**: 1-
5 7, 1993.
6
7
8
9
10 29. Jagtap P, Soriano FG, Virag L, Liaudet L, Mabley J, Szabo E, Hasko G, Marton A,
11 Lorigados CB, Gallyas F, Jr., Sumegi B, Hoyt DG, Baloglu E, VanDuzer J,
12 Salzman AL, Southan GJ and Szabo C, Novel phenanthridinone inhibitors of poly
13 (adenosine 5'-diphosphate-ribose) synthetase: potent cytoprotective and antishock
14 agents. *Crit Care Med* **30(5)**: 1071-82, 2002.
15
16
17
18
19
20
21 30. Jensen AA, Background levels in humans. In: *Halogenated biphenyls, terphenyls,*
22 *naphthalenes, dibenzodioxins and related products* (Eds. Kimbrough RD and
23 Jensen AA), pp. 345-364. Elsevier Science Publishers, 1989.
24
25
26
27
28
29 31. Wassermann M, Wassermann D, Cucos S and Miller HJ, World PCBs map: storage
30 and effects in man and his biologic environment in the 1970s. *Ann N Y Acad Sci*
31 **320**: 69-124, 1979.
32
33
34
35
36 32. Lotti M, Pharmacokinetics and blood levels of polychlorinated biphenyls. *Toxicol*
37 *Rev* **22(4)**: 203-15, 2003.
38
39
40
41 33. Lin CH and Lin PH, Induction of ROS formation, poly(ADP-ribose) polymerase-1
42 activation, and cell death by PCB126 and PCB153 in human T47D and MDA-MB-
43 231 breast cancer cells. *Chem Biol Interact*, 2006.
44
45
46
47 34. Szabo C and Dawson VL, Role of poly(ADP-ribose) synthetase in inflammation
48 and ischaemia- reperfusion. *Trends Pharmacol Sci* **19(7)**: 287-98, 1998.
49
50
51
52 35. Pacher P, Beckman JS and Liaudet L, Nitric oxide and peroxynitrite in health and
53 disease. *Physiol Rev* **87(1)**: 315-424, 2007.
54
55
56
57
58
59
60
61
62
63
64
65

- 1
2
3 36. Walsh T, Donnelly T and Lyons D, Impaired endothelial nitric oxide
4 bioavailability: a common link between aging, hypertension, and atherogenesis? *J*
5 *Am Geriatr Soc* **57**(1): 140-5, 2009.
6
7
8
9
10 37. Yaghoubi M, Oliver-Krasinski J, Cayatte AJ and Cohen RA, Decreased sensitivity
11 to nitric oxide in the aorta of severely hypercholesterolemic apolipoprotein E-
12 deficient mice. *J Cardiovasc Pharmacol* **36**(6): 751-7, 2000.
13
14
15
16
17 38. Ding H, Hashem M, Wiehler WB, Lau W, Martin J, Reid J and Triggle C,
18 Endothelial dysfunction in the streptozotocin-induced diabetic apoE-deficient
19 mouse. *Br J Pharmacol* **146**(8): 1110-8, 2005.
20
21
22
23
24 39. Benko R, Pacher P, Vaslin A, Kollai M and Szabo C, Restoration of the endothelial
25 function in the aortic rings of apolipoprotein E deficient mice by pharmacological
26 inhibition of the nuclear enzyme poly(ADP-ribose) polymerase. *Life Sci* **75**(10):
27 1255-61, 2004.
28
29
30
31
32
33
34 40. Hans CP, Zerfaoui M, Naura AS, Catling A and Boulares AH, Differential effects
35 of PARP inhibition on vascular cell survival and ACAT-1 expression favouring
36 atherosclerotic plaque stability. *Cardiovasc Res* **78**(3): 429-39, 2008.
37
38
39
40
41 41. Pacher P and Szabo C, Role of the peroxynitrite-poly(ADP-ribose) polymerase
42 pathway in human disease. *Am J Pathol* **173**(1): 2-13, 2008.
43
44
45
46 42. Oumouna-Benachour K, Hans CP, Suzuki Y, Naura A, Datta R, Belmadani S,
47 Fallon K, Woods C and Boulares AH, Poly(ADP-ribose) polymerase inhibition
48 reduces atherosclerotic plaque size and promotes factors of plaque stability in
49 apolipoprotein E-deficient mice: effects on macrophage recruitment, nuclear factor-
50
51
52
53
54
55
56
57
58
59
60
61
62
63
64
65

- 1
2
3 kappaB nuclear translocation, and foam cell death. *Circulation* **115**(18): 2442-50,
4
5 2007.
6
7
8 43. Hans CP, Zerfaoui M, Naura A, Troxclair D, Strong JP, Matrougui K and Boulares
9
10 H, Thieno[2,3-c]isoquinolin-5-one (TIQ-A), a potent poly(ADP-ribose) polymerase
11
12 inhibitor, promotes atherosclerotic plaque regression in high fat diet-fed ApoE-
13
14 deficient mice: effects on inflammatory markers and lipid content. *J Pharmacol Exp*
15
16 *Ther*, 2009.
17
18
19 44. Toborek M, Lee YW, Garrido R, Kaiser S and Hennig B, Unsaturated fatty acids
20
21 selectively induce an inflammatory environment in human endothelial cells. *Am J*
22
23 *Clin Nutr* **75**(1): 119-25, 2002.
24
25
26 45. Oliver FJ, J M-dM, Nacci C, Decker P, Andriantsitohaina R, Muller S, de la Rubia
27
28 G, Stoclet JC and de Murcia G, Resistance to endotoxic shock as a consequence of
29
30 defective NF-kappaB activation in poly (ADP-ribose) polymerase-1 deficient mice.
31
32 *Embo J* **18**(16): 4446-54, 1999.
33
34
35
36 46. Andreone TL, O'Connor M, Denenberg A, Hake PW and Zingarelli B, Poly(ADP-
37
38 ribose) polymerase-1 regulates activation of activator protein-1 in murine
39
40 fibroblasts. *J Immunol* **170**(4): 2113-20, 2003.
41
42
43 47. Hasko G, Mabley JG, Nemeth ZH, Pacher P, Deitch EA and Szabo C, Poly(ADP-
44
45 ribose) polymerase is a regulator of chemokine production: relevance for the
46
47 pathogenesis of shock and inflammation. *Mol Med* **8**(5): 283-9, 2002.
48
49
50 48. Piconi L, Quagliario L, Da Ros R, Assaloni R, Giugliano D, Esposito K, Szabo C
51
52 and Ceriello A, Intermittent high glucose enhances ICAM-1, VCAM-1, E-selectin
53
54
55
56
57
58
59
60
61
62
63
64
65

- 1
2
3 and interleukin-6 expression in human umbilical endothelial cells in culture: the role
4 of poly(ADP-ribose) polymerase. *J Thromb Haemost* **2**(8): 1453-9, 2004.
5
6
7
8 49. von Lukowicz T, Hassa PO, Lohmann C, Boren J, Braunersreuther V, Mach F,
9 Odermatt B, Gersbach M, Camici GG, Stahl BE, Tanner FC, Hottiger MO, Luscher
10 TF and Matter CM, PARP1 is required for adhesion molecule expression in
11 atherogenesis. *Cardiovasc Res* **78**(1): 158-66, 2008.
12
13
14
15
16
17 50. Giddings JC, Soluble adhesion molecules in inflammatory and vascular diseases.
18
19 *Biochem Soc Trans* **33**(Pt 2): 406-8, 2005.
20
21
22 51. Lenzser G, Kis B, Snipes JA, Gaspar T, Sandor P, Komjati K, Szabo C and Busija
23 DW, Contribution of poly(ADP-ribose) polymerase to postischemic blood-brain
24 barrier damage in rats. *J Cereb Blood Flow Metab* **27**(7): 1318-26, 2007.
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60
61
62
63
64
65

1
2
3 **Figure legends**
4
5
6

7 **Figure 1. Structure of 2,2',4,6,6'-pentachlorobiphenyl (PCB 104), an example of a**
8 **highly ortho-chlorinated nonplanar PCB.**
9

10
11
12
13
14 **Figure 2. Exposure of *ex vivo* aortic rings to PCB 104 for 4h (A) or 6h (B) dose**
15 **dependently impairs the acetylcholine-relaxant response.** Thoracic aortic rings obtained
16 from male Sprague-Dawley rats were exposed to 1, 3, 10 or 20 μ M PCB 104 for 2, 4 or 6h.
17 Following PCB exposure acetylcholine-induced endothelium-dependent relaxation was
18 determined. Data is expressed as mean \pm SEM from 6 animals; * $p<0.05$, ** $p<0.01$ vs.
19 untreated rings.
20
21
22
23
24
25
26
27

28
29
30
31 **Figure 3. *In vitro* exposure of endothelial cells to PCB 104 dose- and time-**
32 **dependently increased cellular levels of oxidative stress (A), CYP1A1 activity (B), and**
33 **PARP activity (C) while depleting NADPH levels (D).** Mouse endothelial cells were
34 exposed to PCB 104 (1, 3, 10 or 20 μ M) for 2, 4 or 6h prior to the measurements being
35 taken. Data is expressed as mean \pm SEM from 4 separate experiments with 3-6 replicates
36 per experiment; * $p<0.01$ vs. untreated cells.
37
38
39
40
41
42
43
44
45
46
47

48 **Figure 4. PCB 104-mediated impairment of endothelium-dependent aortic ring**
49 **relaxation is prevented by pharmacological inhibition of CYP1A1 or PARP.** Exposure
50 of thoracic aortic rings to 10 μ M PCB 104 for 4h significantly impaired acetylcholine-
51 induced endothelium-dependent relaxation, an effect prevented by simultaneous incubation
52
53
54
55
56
57
58
59
60
61
62
63
64
65

1
2
3 of the rings with the CYP 1A1 inhibitor α -naphthoflavone (NP, 0.3 μ M) or the PARP
4 inhibitor PJ-34 (3 μ M). Data is expressed as mean \pm SEM from 6 animals; **p<0.01 vs.
5 untreated rings, ††p<0.01 vs. PCB 104 treated rings.
6
7
8
9

10
11
12 **Figure 5. Effect of CYP1A1 and PARP inhibition on the PCB 104-mediated**
13 **increase in endothelial cell oxidative stress (A), CYP1A1 activity (B), PARP activity**
14 **(C) and NADPH depletion (D).** Inhibition of CYP1A1 with α -naphthoflavone (0.3 μ M)
15 prevents the increase in oxidative stress, CYP1A1 and PARP activity, plus the NADPH
16 depletion observed following 4h exposure to PCB 104 (10 μ M). Inhibition of PARP either
17 pharmacologically with PJ-34 (3 μ M) or via gene knockout had no effect on PCB-104-
18 mediated increase in oxidative stress or CYP1A1 activity but did inhibit the increase in
19 PARP activity and NADPH depletion. Data is expressed as mean \pm SEM from 4 separate
20 experiments with 3-6 replicates per experiment; *p<0.05, ** p<0.01 vs. untreated cells
21 †p<0.01 vs. PCB 104 treated cells.
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38

39 **Figure 6. Exposure of endothelial cells to PCB 104 dose-dependently reduced cell**
40 **viability, an effect attenuated by inhibition of CYP1A1 and PARP either**
41 **pharmacologically or through gene deletion.** Data is expressed as mean \pm SEM from 4
42 separate experiments with 3-6 replicates per experiment; *p<0.05, ** p<0.01 vs. untreated
43 cells †p<0.01 vs. PCB 104 treated cells.
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60
61
62
63
64
65

Exposure time	4h	6h
Control	$1.1 \times 10^{-7} \pm 0.2 \times 10^{-7}$ M	$0.8 \times 10^{-7} \pm 0.2 \times 10^{-7}$ M
PCB 104 3 μ M	$2.0 \times 10^{-7} \pm 0.6 \times 10^{-7}$ M*	$5.6 \times 10^{-7} \pm 3.3 \times 10^{-7}$ M*
PCB 104 10 μ M	$4.1 \times 10^{-7} \pm 1.2 \times 10^{-7}$ M*	$8.3 \times 10^{-7} \pm 3.7 \times 10^{-7}$ M*
PCB 104 20 μ M	$9.4 \times 10^{-7} \pm 1 \times 10^{-7}$ M*	$15.4 \times 10^{-7} \pm 7.7 \times 10^{-7}$ M*

Table 1 Exposure of *ex vivo* aortic rings to PCB 104 for 4h or 6h increases the EC50 of acetylcholine. Thoracic aortic rings obtained from male Sprague-Dawley rats were exposed to 3, 10 or 20 μ M PCB 104 for 4 or 6h. Following PCB exposure acetylcholine-induced endothelium-dependent relaxation was determined and EC 50 measured. Data is expressed as mean \pm SEM from 6 animals; * $p < 0.05$ vs. untreated rings.













